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Sodium and Chloride Transport in the Lizard Duodenum

Anthonio Lorenzo[1], Pino Santana, Tomas Gómez and Pilar Badia

Laboratorio de Fisiologia Animal, Departamento de Biologia Animal, Facultad de Biologia, Universidad de La Laguna, Tenerife, España

ABSTRACT—The mechanisms by which Na and Cl ions are transported across the duodenal epithelium of the lizard *Gallotia galloti* were studied under voltage-clamped conditions. The lizard duodenum actively absorbed sodium and chloride with a ratio of approximately 2:1. In the absence of sodium, the short-circuit current (Isc) and net chloride flux (J^{Cl}_{net}) were abolished. In the absence of chloride, the net sodium flux (J^{Na}_{net}) was halved but the short-circuit current (Isc) was not changed significantly. Treatment with acetazolamide, amiloride, or disulfonic stilbene (DIDS) abolished the net chloride flux almost completely, whereas the Isc was not changed significantly. When ouabain was added to the serosal side, the short-circuit current and net fluxes of sodium and chloride were abolished. From an analysis of the effects of these inhibitors, a plausible model was developed to explain the characteristics of the sodium and chloride transport. It is proposed that the entry of sodium into the cell across the luminal membrane occurs via two pathways. A part of the Na^+ entry occurs through the Na^+/H^+ antiporter and a part through an electrogenic pathway. The entry of Cl^- across the apical membrane occurs through the Cl^-/HCO_3^- antiport. Chloride seems to exit the cell by a diffusional process.

INTRODUCTION

Electrolyte transport across leaky epithelia such as the gallbladder, renal proximal tubule and small intestine has been studied extensively *in vivo* and *in vitro* by measuring electrical parameters and tracer fluxes [1–3]. Numerous studies have demonstrated an interdependence of net transepithelial Na^+ and Cl^- fluxes [4–7]. These results have been interpreted by the existence of direct coupling of Na^+ and Cl^- fluxes through a ternary-complex of Na^+ and Cl^-, and cotransporter at the apical membrane [2]. However, the other possibility of neutral, coupled NaCl transport resulting from the simultaneous operation of Na^+/H^+ and Cl^-/OH^- exchanges has been suggested [8–10]. These transport mechanisms have been studied specially in the small intestine of mammals and few references are found in reptilian intestine, where electrolyte transport has been studied especially in the colon. Studies of the proximal and distal colon of *Testudo graeca* [11] have demonstrated that sodium and chloride are transported at a similar rate. Active transport of sodium and chloride ions has also been shown across the isolated turtle colon [12], lizard colon [13], and ileum [14].

The purpose of the present study was to investigate mechanisms of Cl^- and Na^+ transport across isolated lizard duodenum by measuring unidirectional sodium and chloride fluxes in presence of several inhibitors. The results suggest that about 50% of apical sodium entry results from amiloride sensitive Na^+/H^+ exchange and that of the other 50% of the sodium uptake is electrogenic. The entry of chloride into the cell across the luminal membrane results from a Cl/HCO_3 exchange.

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[1] To whom reprint requests should be addressed.

MATERIALS AND METHODS

Animals and preparation

Experiments were performed on male and female *Gallotia galloti* lizards (mean body weight

30–40 g). They were killed by spinal transection and ileum was removed and placed in ice cold bathing solution gassed with 5% CO_2 in O_2. Duodenal mucosa was separated from underlying smooth muscle and was mounted in Ussing chambers with an exposed area of 0.21 cm^2. Chambers were water jacketed and the temperature was maintained at 30°C. The tissue was bathed on both sides with each experimental solution (4.0 ml).

Solutions

The bathing solution contained in mM: NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1.0 and glucose 5. The solution was gassed with 5% CO_2 in O_2 and pH of 7.4. In Na^+-free solution, Na^+ was replaced with choline. When Cl^--free solution was used, Cl^- was replaced with isothionate. HCO_3-free solution was buffered with Hepes-Tris (pH= 7.4) and gassed with pure O_2.

Electrical measurements

Agar bridges, 4% w/v made with bathing solution, were positioned near each of the tissues and at opposite ends of the chamber. Calomel electrodes and Ag/AgCl electrodes in saturated KCl were connected to the agar bridges to measure the transmural potential difference and to pass direct current, respectively. The tissue was continuously short circuited with an automatic computer controlled voltage clamp device (AC-Microclamp, Aachen, W. Germany). Algorithm A6 was utilized. Briefly, the procedure was as follows: after correcting for offset potential and solution resistance, direct current was applied to nullify the transmural potential difference. The necessary current (Isc) was corrected at a frequency of 4Hz. Every 5 sec, a 25 μA pulse (1 sec duration) was alternately added, which was substracted from the short-circuit current (Isc). After a 0.5 sec delay, the displacement in potential difference from zero caused by the pulse was measured. From the change in potential difference and pulse amplitude, tissue conductance (Gt) was calculated. The transmural open circuit potential difference (PD) was calculated from the Gt and Isc. All three parameters, Isc, Gt and PD were recorded by a digital printer at 1 min intervals. In addition, Isc was continuously recorded on a chart recorder.

Unidirectional ion flux measurements

Twenty min after the tissue was mounted, isotopes (^{22}Na and ^{36}Cl) were added to the bathing solution on one side of the tissue. After additional 20 min, by which time isotope fluxes had reached a steady-state, samples were taken from the unlabelled side every 20 min. After each sampling, equal volume of cold solution was added to maintain the volume. The effects of inhibitors on ion fluxes were studied by comparing ion fluxes before (3 samples) and after (3 samples) addition of each inhibitor. Isotope activities were determined simultaneously by sequential counting in a Beckman liquid scintillation spectrometer (Nuclear Chicago, model Isocap-300). Unidirectional Na^+ and Cl^- fluxes were calculated using standard equations [15]. The net residual ion flux (J^R_{net}) was calculated from the difference in Isc and net Na^+ ($J^{Na^+}_{net}$) and Cl^- ($J^{Cl^-}_{net}$) fluxes (J^R_{net})=Isc− ($J^{Na^+}_{net}$)−($J^{Cl^-}_{net}$).

Statistics

Results are given as the mean ± one standard error of the mean (SEM). Significances of differences were tested using a two-tailed Student's *t*-test. Paired or unpaired tests were used.

Materials

Amiloride, acetazolamide, DIDS (4-4′-diisothio- cyanatostilbene-2-2′-disulfonic acid) and ouabain were obtained from Sigma Chemical (St. Louis). Radioisotopes (^{22}Na and ^{36}Cl) were obtained from New England Nuclear.

RESULTS

Electrical and flux measurements under standard conditions

The isolated duodenum was first incubated for 40 min (t_0-t_{40}) under open-circuit conditions and then for 60 min (t_{40}–t_{100}) under short-circuit conditions (Fig. 1). Under open-circuit conditions the measured PD (serosa positive) and Gt and the calculated Isc were equal to the respective calculated PD and measured Gt and Isc under short-

circuit conditions. Thus the short-circuiting does not appear to affect the duodenum from lizards. Measurements of Na^+ and Cl^- fluxes across the short-circuited mucosa are given in Figure 1. Net fluxes of Na^+ and Cl^- were observed under short-circuited condition, whereas Na^+ absorption predominated. The J^{Na}_{net} was already twice times the J^{Cl}_{net} ($J^{Na}_{net}=2.35\pm0.16$ and $J^{Cl}_{net}=1.25\pm0.15$ μeq/cm^2 h) and the difference between them was approximately equal to the Isc and the residual flux did not differ significantly from zero.

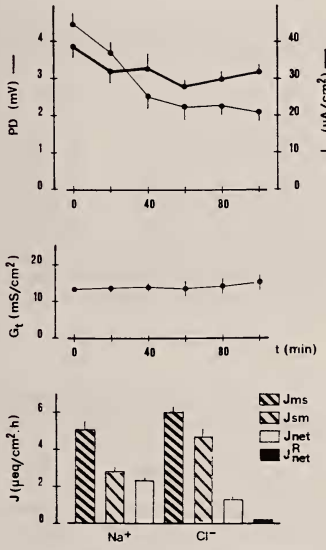

FIG. 1. Electrical characteristics and sodium and chloride fluxes in lizard duodenum under standard conditions. The bars represent SEM of 14 experiments.

Effects of substitutions on the electrical characteristics and ion fluxes

In order to test the coupling between sodium and chloride transport processes, Cl^- and Na^+ fluxes were determined in sodium-free and chloride-free Ringer solutions respectively. The tissue was first incubated in standard bathing solution in order to obtain control values of electrical parameters and unidirectional fluxes. The tissue was then rinsed three times with either a Na^+-free solution containing the normal concentration of Cl^- (114 mM) or a Cl^--free solution containing the normal concentration of Na^+ (136 mM) and incubated until a new steady-state was obtained.

When Na ions were replaced with choline Isc and PD were decreased to zero ($Isc=-0.006\pm0.15$ μequ/cm^2 h, $PD=-0.15\pm0.31$ mV), the tissue conductance remaining unchanged ($Gt=18.25\pm2.75$ mS/cm^2). In contrast, the PD and Isc, in isothionate Ringer were not significantly different from control values ($PD=3.26\pm0.35$ mV, $Isc=1.03\pm0.12$ μeq/cm^2 h, $Gt=11.45\pm2.80$ mS/cm^2). Steady-state Na^+ and Cl^- fluxes in Cl^--free and Na^+-free Ringer's solution are given in Table 1. Na^+ replacement with choline (Na-free) abolished J^{Cl}_{net} and Cl^- replacement with isothionate (Cl-free) halved J^{Na}_{net}. This appears to indicate that approximately 50% of the net sodium transport is coupled with the net Cl^- transport.

Effects of various inhibitors, acetazolamide, amiloride and ouabain, on ion fluxes

In order to determine whether NaCl absorption is via Na^+/Cl^- cotransport or Na^+/H^+-Cl^-/HCO_3^- dual exchange system, the effects of acetazolamide, amiloride and ouabain on the Na^+ and Cl^- fluxes were investigated. The results are listed in Table 2. Working with HCO_3^--free solutions, net sodium and cloride fluxes did not change significantly with respect to control solution. When acetazolamide was added to both mucosal and serosal bathing solutions (HCO^{3-}-free), the net chloride transport was completely inhibited (-0.47 ± 0.18 μeq/cm^2 h) due to a decrease in the mucosa to serosa chloride flux. Net codium transport was almost halved. The Isc, PD and Gt remained unchanged. This appears to indicate that chloride absorption is all via Cl^-/HCO_3^- antiport and 50% of sodium absorption is via Na^+/H^+ antiport and the electrical parameters are due to the remaining 50% Na^+ transport.

The addition of amiloride (10^{-3} M) to the mucosal side (standard solution) revealed that this substance completely inhibited the net chloride transport (0.07 ± 0.17 μeq/cm^2 h) and halved the

Table 1. Effects of ion substitution on electrical

	J^{Na} (n=14)			J^{Cl} (n=13)		
	ms	sm	net	ms	sm	net
Control	5.5 ±0.57	3.2 ±0.31	2.29±0.17	6.27±0.36	5.11±0.47	1.16±0.16
Cl-free	4.30±0.60*	3.18±0.59	1.12±0.22*	—	—	—
Na-free	—	—	—	5.30±0.11†	5.39±0.54	−0.09±0.15*

Values are means±SEM, n=number of tissues.
Ion fluxes and ISC are given in μeq/cm^2 h, PD in mV and Gt in mS/cm^2 h. Measurements were made before conditions the chloride ions of both sides of the tissue were replaced by isothionate; likewise, the sodium-Na-free conditions.
†,*significant difference ($P<0.05$, $P<0.001$) respectively, with the control series according to t-test.

Table 2. Effects of acetazolamide, amiloride and ouabain

		J^{Na}			J^{Cl}		
	(n)	ms	sm	net	ms	sm	net
(HCO_3^--free)	(10)	6.50±0.57	3.21±0.31	2.29±0.20	7.23±0.25	5.87±0.36	1.36±0.13
Acetazolamide	(10)	4.50±0.30#	3.60±0.38	0.90±0.12*	5.68±0.47#	6.15±0.34	−0.47±0.18*
Control	(14)	8.39±0.55	6.25±0.42	2.14±0.18	9.31±0.40	8.20±0.60	1.11±0.19
Amiloride	(14)	7.50±0.49#	6.25±0.30	1.15±0.15*	8.10±0.39†	8.03±0.50	0.07±0.17*
Control	(11)	5.60±0.40	3.10±0.61	2.50±0.21	6.32±0.35	5.46±0.41	0.86±0.16
Ouabain	(11)	4.70±0.50#	4.70±0.45†	0.00±0.20*	5.20±0.13#	5.38±0.32	−0.18±0.09*

Ion fluxes and Isc are given in μeq/cm^2 h, PD in mV and Gt in mS/cm^2. Measurements were made before and after Acetazolamide was added to the HCO_3^--free bathing solution both sides of tissue. Amiloride and ouabain tively. Values are the mean±SEM.
†,#,*sigmificant difference $P<0.05$, $P<0.01$, $P<0.001$.

Table Effects of DIDS on ion fluxes

		J^{Na}			J^{Cl}		
	(n)	ms	sm	net	ms	sm	net
Control	(9)	6.25±0.68	3.90±0.43	2.35±0.25	6.32±0.48	5.02±0.89	1.30±0.34
DIDS (mucosa)	(9)	4.08±0.37	2.67±0.25	1.41±0.15#	4.96±0.41†	4.78±0.79	0.18±0.30
Control	(9)	6.70±0.56	4.20±0.28	2.50±0.21	6.15±0.42	5.20±0.63	0.95±0.25
DIDS (Serosa)	(9)	5.60±0.28	3.40±0.43	2.20±0.17	6.08±0.43	5.16±0.45	0.91±0.20

Ion fluxes and Isc are given in μeq/cm^2 h, PD in mV and Gt in mS/cm^2. Measurements were made before and after Values are the mean±SEM.
†,#significant difference ($P<0.05$, $P<0.01$) compared to control.

net sodium transport while Isc, PD and Gt remained unchanged. This observation indicates that amiloride inhibits the Na^+/H^+-Cl^-/HCO_3^- dual exchange system across the apical membrane of the epithelial cell. The effects of ouabain (10^{-3} M) added to the serosal side showed that sodium and chloride transport was dependent on the activity of the basolateral Na^+-K^+ pump. As indicated in Table 2, the Na^+ and Cl^- transport and Isc were all abolished.

characteristics and ion fluxes

(n=14)		
Isc	PD	Gt
1.24 ±0.18	2.99±0.15	12.07±1.86
1.03 ±0.12	3.26±0.35	11.45±2.80
−0.006+0.15*	−0.15±0.31*	18.15±2.75

and after the substitution of the ion. Under the Cl-free ions were substituted in both media by choline under the

on ion fluxes in duodenum

Isc	Gt	PD
1.13±0.15	12.15±1.82	2.10±0.28
1.07±0.12	11.42±2.02	2.22±0.18
1.14±0.12	13.15±2.65	2.39±0.23
1.10±0.23	13.14±1.67	2.27±0.19
1.42±0.30	17.68±2.68	2.54±0.23
0.49±0.16*	26.00±4.08	2.21±0.22

the addition of inhibitors to the bathing solution. were only added to the mucosal and serosal side respec-

in duodenum

Isc	Gt	PD
1.23±0.16	18.06±2.06	1.92±0.23
1.08±1.12	21.56±2.44	1.35±0.19
1.26±0.17	13.20±1.99	2.21±0.22
1.32±0.23	15.54±1.26	2.18±0.30

the addition of inhibitor to the bathing solution.

Effects of DIDS on ion fluxes

The disulfonic stilbenes SITS and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) have been utilized as anion exchange inhibitors [10]. To further differentiate the chloride transport across the brush-border membrane from that across the basolateral membrane, we next examined the effects of DIDS on Na^+ and Cl^- fluxes. As indicated in Table 3, after addition of 10^{-3} M DIDS to the mucosal bath, Isc, PD and Gt remained unchanged, but DIDS reduced the net flux of sodium by decreasing the m-s flux and abolished the net flux of chloride. These results suggest, as indicated above, that chloride absorption is via Cl^-/HCO_3^- and a part of sodium absorption is via Na^+/H^+-Cl^-/HCO_3^- dual exchange system. When DIDS was added to serosal side the mucosal to serosal chloride flux was not reduced at all (Table 3), suggesting that chloride exit does not occur through a Cl^-/HCO_3^- exchange.

DISCUSSION

Several reports suggest an obligatory coupling of the transport of sodium and chloride across the brush-border membrane of leaky epithelia [1, 2, 6, 16–24]. Recently a significant contribution of a Na^+/Cl^- cotransport mechanism to NaCl uptake across the intestinal brush-border membranes has been excluded in rat small intestine [9, 10]. The findings are, consistent with a double exchange of Na^+ for H^+ and Cl^- for OH^- (HCO_3^-). On the other hand, studies performed in apical membrane of *Necturus* gallbladder [25] also support the hypothesis that NaCl entry results primarily from the operation of parallel Na^+/H^+ and Cl^-/HCO_3^- exchanges, and not from a NaCl cotransport.

The importance of the present results lies in how they relate to the mechanism of NaCl entry into lizard duodenal epithelium. *Gallotia* duodenal mucosa *in vitro* develops a small transmural potential and high tissue conductance, which are characteristics of leaky epithelia. Under control conditions, duodenum actively absorbs sodium and chloride in a stoichiometric ratio of 2:1. These results differ from those described for other leaky epithelia, where the net fluxes of sodium and chloride are identical under the steady state [25, 26].

The discrepancy between the net sodium flux and the short circuit current may mean that a part of the net sodium transport is due to an electrically neutral mechanism or that other electrogenic

mechanism opposite in sign contributes to the Isc. When chloride was replaced with isothionate (Table 1) the J_{ms} and J_{net} of sodium were significantly reduced. It is important to point out that the net flux was not abolished but was, in fact reduced by 50%. When sodium was replaced by chloride, J_{ms}^{Cl} was reduced significantly; the amount of the reduction (0.97 μeq/cm^2 h) was similar to the amount by which J_{ms}^{Na} was reduced (1.2 μeq/cm^2 h) in the absence of chloride. These results indicate that a coupled transport exists, which accounts for approximately 50% of the net sodium transport and all of the net chloride transport. The fact that the I_{mc} was near zero in the absence of sodium seems to be consistent with the existence of a sodium electrogenic transport, responsible for the Isc.

One method of deciding whether the transport of NaCl is via Na^+-Cl^- cotransport or Na^+/H^+ Cl^-/HCO_3^- dual exchange system would be to study the transport at zero CO_2 and HCO_3^- concentrations. The intracellular absence of HCO_3^- and H^+ would block the mechanisms of Cl^-/HCO_3^- and Na^+/H^+. Our results show that in HCO_3^--free solution and in presence of acetazolamide, the net sodium flux was inhibited by 50% and the net chloride flux was abolished completely. Short circuit current was not affected by the acetazolamide. This suggests that all of the net chloride transport takes place via Cl^-/HCO_3^- exchange and 50% of the sodium via Na^+/H^+ exchange.

Other evidence for Na^+/H^+-Cl^-/HCO_3^- transport and against Na^+-Cl^- contransport was the use of specific inhibitors such as amiloride and DIDS. Amiloride has been described as an inhibitor of the Na^+/H^+ antiport for a wide variety of epithelia [27] and as an inhibitor of the sodium channels in the apical membrane of tight epithelia at lower concentration [28]. The addition of amiloride to the mucosal solution abolished the net chloride flux and halved the net sodium flux across the tissue. The PD and Isc were not significantly reduced. These results suggest that amiloride blocks the Na^+/H^+ anitiport, it would reduce the intracellular pH and abolishes the formation of HCO_3^- and therefore inhibits the Cl^-/HCO_3^- transport system. Amiloride did not block the electrogenic sodium transport since the Isc did not change.

The effects of DIDS support the hypothesis described above for NaCl transport mechanisms. DIDS has been reported as a potent inhibitor of the Cl^-/HCO_3^- exchange in red blood cells [29] and in other cell types such as turtle bladder [30] and muscle fiber [31]. When DIDS was added to the mucosal side, the effect was similar that obtained after the treatment with amiloride; the net chloride flux was abolished and the net sodium flux was halved. If DIDS is a specific inhibitor of Cl^-/HCO_3^-, these results support the hypothesis that the mechanism of NaCl transport across lizard duodenum is via dual exchange mechanism of Na^+/H^+ and Cl^-/HCO_3^-, similar to the model proposed for human ileum [8], rat small intestine [10] and rat kidney [32].

The energy for entry of NaCl derives from two sources: the Na^+/K^+ ATPase, that allows a large potential energy to be made as the Na^+ gradient across the mucosal membrane and the other from the production of HCO_3^- and H^+ from CO_2 and H_2O inside the cell. If the production of H_2CO_3 ($H^+ + HCO_3^-$) is abolished by inhibiting carbonic anhydrase with acetazolamide, the Na^+/H^+-HCO_3^-/Cl^- dual exchange system will be inhibited. If the Na^+/K^+ ATPase is blocked by ouabain, then all the Na^+-dependent transport systems would cease since the blockage of the Na^+-K^+ pump would increase the intracellular Na^+ concentration. The increase in the intracellular Na^+ will inhibit both the electrogenic sodium transport and the Na^+/H^+ antiport. The results obtained from ouabain treatment (Table 2) clearly indicate that sodium and chloride transport depends on Na^+/K^+ ATPase activity, which is consistent with a consideration cited above.

Figure 2 shows a possible model which could account for the various features observed in the present study. The entry of sodium into the cell across the luminal membrane occurs by two pathways. A part of the influxes occurs through the Na^+/H^+ antiport and a part through an electrogenic conductive pathway. The second pathway may be responsible for the serosa-positivity of the PD under standard conditions. The chloride ion would enter across the brush border membrane in

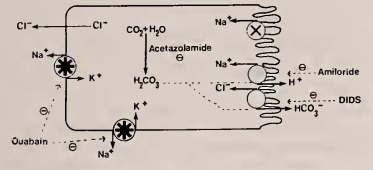

FIG. 2. Provisional model for ion movements across the lizard duodenum epithelium. For details see text.

exchange for bicarbonate. The Na^+/K^+ ATPase is responsible for sodium exit through the basolateral membrane. Since DIDS in serosal bathing solution did not reduce the chloride fluxes, chloride exit through the basolateral membrane does not seem to be via Cl^-/HCO_3^- exchange but due to a passive diffusion. This model is similar to that for the ileum of lizard [14].

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